VIII-3-1	**Declaration: Entitlement to claim priority** Declaration as to the applicant's entitlement, as at the international filing date, to claim the priority of the earlier application specified below, where the applicant is not the applicant who filed the earlier application or where the applicant's name has changed since the filing of the earlier application (Rules 4.17(iii) and 51bis.1(a)(iii)): Name:	in relation to this international application NEUROGEN CORPORATION is entitled to claim priority of earlier application No. 60/413,321 by virtue of the following:
VIII-3-1 (iv)		an assignment from RAJACHANDRAN, Lavanya to NEUROGEN CORPORATION, dated 02 October 2002 (02.10.2002)
VIII-3-1 (iv)		an assignment from BERETTA, Elena to NEUROGEN CORPORATION, dated 03 October 2002 (03.10.2002)
VIII-3-1 (iv)		an assignment from KRAUSE, James to NEUROGEN CORPORATION, dated 02 October 2002 (02.10.2002)
VIII-3-1 (ix)	This declaration is made for the purposes of:	all designations

P1 1089963

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office

November 06, 2003

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE.

APPLICATION NUMBER: *60/413,321*
FILING DATE: *September 25, 2002*
RELATED PCT APPLICATION NUMBER: *PCT/US03/29916*

By Authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS

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09-25-02 60413321.092502 A/P

Attorney Docket No: NO2.2100P

jc944 U.S. PTO
09/25/02

PATENT
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

jc996 U.S. PTO
60/413321
09/25/02

In Re Application of: Rajachandran et al.

Serial No. : Not yet assigned — Examiner: Not yet assigned

Filed: Herewith

For: Methods for Treating Obesity in Patients with MC4 Receptor Mutations — Art Unit: Not yet assigned

Box PROVISIONAL APPLICATION
Assistant Commissioner for Patents
Washington, D.C. 20231

COVER SHEET FOR FILING PROVISIONAL PATENT APPLICATION

US Express Mailing No. EF307729707US

Dear Sir:

The accompanying application, entitled" Methods for Treating Obesity in Patients with MC4 Receptor Mutations," is a provisional patent application under 37 C.F.R. § 1.51 (a)(2) and § 1.53 (b)(2).

1. ☒ The names and addresses of the inventors of this application are as follows:

	Last Name	First Name	Middle Initial	Residence
1.	Rajachandran	Lavanya		53 Cliffside Drive Wallingford, CT 06492
2.	Beretta	Elena		93 Florence Road, apt # 1B Branford, CT 06405
3.	Krause	James	E.	123 Five Field Rd Madison, CT 06443

2. ☐ This invention was made by an agency of the United States Government or under contract with an agency of the United States Government. The name of the U.S. Government agency and the Government contract number are:

Agency: _____

Contract No.: _____

[redacted]

3. The following documents are enclosed:

- ☒ 38 pages of specification
- ☒ 1 page of abstract
- ☐ _____ pages of drawings

4. ☐ A verified statement to establish small entity status under 37 CFR 1.9 and 1.27 is enclosed.

5. ☐ An Assignment of the invention to _____ is enclosed. A check in the amount of $40.00 for recording this assignment and a recordation form cover sheet (Form PTO 1595) are also enclosed.

6. ☒ The fee for filing this provisional application, as set forth in 37 CFR 1.16(k), is $80.00.

   a. ☒ **A check for this filing is enclosed.**

   b. ☐ Charge the filing fee to Deposit Account No. 501116.

   c. ☐ The filing fee is not being paid at this time.

7. ☒ Please charge any fee deficiencies associated with this filing to Deposit Account No. 501116. *A duplicate copy of this sheet is enclosed.*

Please address all future communications to:

Patent Department
Neurogen Corporation
35 Northeast Industrial Road
Branford, CT 06405

and direct telephone calls to: Seth A. Fidel, Leslie-Anne Horvath or Ann Kadlecek.

Respectfully submitted,

9/25/2002
Date

Ann Kadlecek
Registration No. 39,244

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Neurogen Corporation
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203-488-8201

Atty. Docket No: N02.2100P

PATENT
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:	Rajachandran et al.	
Serial No. :	Not yet assigned	Examiner: Not yet assigned
Filed:	Herewith	
For:	Methods for Treating Obesity in Patients with MC4 Receptor Mutations	Art Unit: Not yet assigned

Box PROVISIONAL APPLICATION
Assistant Commissioner for Patents
Washington, D.C. 20231

EXPRESS MAIL INFORMATION

Attached hereto are the following papers which are to being sent by Express Mail Post Office To Addressee Service to: Box Provisional Application, Assistant Commissioner for Patents, Washington, D.C., 20231 on September 25, 2002, with Express Mail Mailing No. EF307729707US:

1. Provisional Application for Patent Cover Sheet (2 sheets, 2 copies)
2. Specification for Patent Application: Methods for Treating Obesity in Patients with MC4 Receptor Mutations (38 pages)
3. Abstract for patent application (1 page)
4. Check for Provisional Application Filing Fee (1 check, $80.00)
5. Return Postcard

Respectfully submitted:
Ann Kadlecek
Reg. No. 39,244
Neurogen Corp.
35 NE Industrial Rd.
Branford, CT 06405

9/25/2002
Date

Signed

[redacted]

PROVISIONAL APPLICATION FOR UNITED STATES LETTERS PATENT IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Title: Methods for Treating Obesity in Patients with MC4 Receptor Mutations

Inventors:

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Methods for Treating Obesity in Patients with MC4 Receptor Mutations

Field of the Invention

This invention relates generally to methods for treating health conditions associated with altered MC4 receptor activity, and more specifically to the use of melanin concentrating hormone receptor antagonists for the treatment of obesity and overeating in patients carrying MC4 receptor mutations.

Background of the Invention

Obesity is the most common nutritional problem in developed countries. By some estimates, obesity affects more than half of the population of the United States, where about 300,000 deaths annually are attributable to this condition. Obesity often leads to serious health conditions, such as diabetes, atherosclerosis, pulmonary embolism, coronary artery disease, hypertension, stroke, diabetes, sleep apnea, deep-vein thrombosis, hyperlipidemia and some cancers, and complicates numerous chronic conditions such as respiratory diseases, osteoarthritis, osteoporosis, gall bladder disease and dyslipidemias. Fortunately, however, many of the conditions caused or exacerbated by obesity can be resolved or dramatically improved by weight loss.

Once considered merely a behavioral problem (*i.e.*, the result of voluntary hyperphagia), obesity is now recognized as a complex multifactorial disease involving defective regulation of food intake, food-induced energy expenditure and the balance between lipid and lean body anabolism. Both environmental and genetic factors play a role in the development of obesity. As a result, treatment programs that focus entirely on behavior modification have limited efficacy and are associated with recidivism rates exceeding 95%. Pharmacotherapy is now seen as a critical component of weight loss and subsequent weight management.

The central melanocortin system is critical for the regulation of food intake and energy balance. Within this system, melanocortins (a variety of different peptide products resulting from post-translational processing of pro-opiomelanocortin) stimulate or inhibit food intake via action at one or more melanocortin receptors. Alterations in melanocortin receptor activity have been shown to affect food intake.

Five melanocortin receptor subtypes have been described to date. Of these, melanocortin 4 receptor (MC4R) is the most abundant and most widely distributed in the brain. MC4R plays a specific role in appetite regulation in humans and contributes significantly to genetic causes of

obesity. Most known genetic mutations that result in obesity are recessive and cause only rare forms of obesity that occur in combination with endocrine abnormalities. Mutations in MC4R, however, can be dominant and are the most frequent known cause of severe obesity, estimated to occur in 3-5% of obese patients. MC4R is a 332-amino acid protein that belongs to the family of seven transmembrane G protein-coupled receptors (GPCR) and signals via adenylate cyclase. This receptor is expressed primarily throughout the brain, and is activated by the melanocortin alpha-melanocyte stimulating hormone (alphaMSH). In both humans and mice, interruption of signaling at MC4R increases overeating, body-mass index and obesity, and a variety of mutations in MC4R have been associated with human obesity. MC4R agonists have been shown to reduce food intake, while antagonists of this receptor stimulate food intake.

Other metabolic pathways also appear to contribute to obesity. Melanin concentrating hormone, or MCH, is a cyclic 19 amino acid hypothalamic neuropeptide that functions as a regulator of food intake and energy balance, serving as a neurotransmitter in the lateral and posterior hypothalamus. MCH mRNA is overexpressed in ob/ob C57BL/6J mice, and mice with a targeted deletion of the MCH gene are characterized by reduced body weight, due to decreased feeding and increased metabolic rate. ICV administration of MCH has been shown to produce a mild orexigenic effect

MCH activity is mediated via binding to specific receptors, of which MCH type 1 and type 2 receptors have been identified. MCHR1 is a 353 amino acid, 7-transmembrane, alpha-helical, G-coupled protein receptor, initially reported by Kolakowski et al. (1996) *FEBS Lett. 398*:253-58; Lakaye et al. (1998) *Biochim. Biophys. Acta 1401*:216-220; Chambers et al. (1999) *Nature 400*:261-65; and Saito et al. (1999) *Nature 400*:265-69. Upon binding MCH, MCHR1 receptors expressed in HEK 293 cell mediate a dose dependent release of intracellular calcium. Cells expressing MCH receptors have also been shown to exhibit a pertussis toxin sensitive dose-dependent inhibition of forskolin-elevated cyclic AMP, indicating that the receptor couples to a $G_{i/o}$ G-protein alpha subunit. MCHR2 (An et al. (2001) *Proc. Natl. Acad. Sci. USA 98*:7576-7581; Sailer et al. (2001) *Proc. Natl. Acad. Sci. USA 98*:7564-7569; Hill et al. (2001) *J. Biol. Chem. 276*:20125-20129; Mori et al. (2001) *Biochem. Biophys. Res. Commun. 283*:1013-1018) has an overall amino acid identity of more than 30% with MCHR1, and is detected in most regions of the brain, with an expression pattern similar to that of MCHR1.

Although multiple neurotransmitter and hormonal pathways that contribute to obesity are known, the relationships among pathways are poorly understood. As a result, attempts to identify agents that decrease food intake in patients with MC4R mutations have focused on the identification of agents that act specifically at MC4R to increase activity or expression. To date,

however, no such agents are commercially available. The current limited understanding of the molecular and genetic contributions to obesity has hampered the search for effective agents capable of inhibiting food intake in individuals with diminished MC4 receptor activity.

Accordingly, there is a need in the art for agents, especially small molecule, non-peptide agents, that are capable of inhibiting food intake in individuals with diminished MC4 receptor activity. The present invention fulfills this need, and provides further related advantages.

Description of the Sequence Listing

SEQ ID NO:1	Cynomolgus macaque MCH1R DNA sequence
SEQ ID NO:2	Cynomolgus macaque MCH1R amino acid sequence
SEQ ID NO:3	5' Cynomolgus macaque MCH1R primer
SEQ ID NO:4	3' Cynomolgus macaque MCH1R primer
SEQ ID NO:5	MC4R amino acid sequence (Gantz et al. (1993) *J. Biol. Chem.* *268*:15174-79)

Summary of the Invention

The present invention provides compositions and methods useful for the treatment of overeating and obesity in patients carrying a MC4R mutation. Compositions generally comprise an effective amount of one or more MCH receptor antagonists, in combination with a physiologically acceptable carrier or excipient.

Within certain aspects, the present invention provides methods for treating obesity in a mammalian patient, comprising determining whether or not the patient carries a melanocortin 4 receptor (MC4R) mutation and, if the patient carries such a mutation, administering an amount of a non-toxic melanin concentrating hormone (MCH) receptor antagonist effective to reduce the body mass index of the patient upon repeated administration.

Within further aspects, the present invention provides methods for treating obesity in a patient with a MC4R mutation, comprising administering an effective amount of a non-toxic MCH receptor antagonist to a patient previously determined to carry sucha mutation.

These and other aspects of the present invention will become apparent upon reference to the following detailed description.

Detailed Description of the Invention

As noted above, the present invention provides compositions and methods for use in treating patients with MC4R mutations. Compositions provided herein generally comprise a

non-toxic MCH receptor antagonist. Such compositions may be administered to a patient with an MC4R mutation, for example, to reduce food intake, BMI and/or obesity.

TERMINOLOGY

A "patient" is any individual being considered for treatment with an MCH receptor antagonist. Patients include humans, as well as other mammals such as companion animals and livestock, and are preferably obese.

A "melanocortin 4 receptor (MC4R) gene" is a naturally-occurring nucleotide sequence that encodes a MC4R (*i.e.*, a G-protein coupled receptor that comprises an amino acid sequence that is at least 90% identical to SEQ ID NO:5). The encoded MC4R sequence may be truncated relative to SEQ ID NO:5; in such cases, the percent identity is determined using only the portion of SEQ ID NO:5 that aligns with the MC4R encoded by the patients MC4R gene using, for example, a ClustalW alignment. The term "MC4R gene" encompasses both the coding region and any introns or upstream or downstream regions that are tightly linked to the MC4R locus.

Patients are said to "carry a MC4R mutation" if the nucleotide sequence of one or both of the patient's MC4R genes contains at least one sequence feature that, prior to considering the patient for treatment, is determined to be associated with obesity. Each sequence feature associated with obesity is referred to herein as a MC4R mutation, and may be located in an upstream region, coding region, intron or downstream region. A MC4R mutation is generally a sequence alteration (*e.g.*, any nucleotide deletion, insertion, or substitution) or other modification (*e.g.*, an altered methylation state) relative to a reference MC4R sequence for a non-obese member of the patient's species. An appropriate reference sequence for humans is the MC4R sequence available at GenBank Accession Number L08603, a translation of which is provided herein as SEQ ID NO:5, and appropriate reference sequences for other animals may be obtained using conventional molecular biological techniques, using the human sequence as a probe. A determination as to whether a patient carries an MC4R mutation may be performed using standard techniques, such as PCR or RFLP mapping, with or without isolation of the MC4R gene. If prior genetic testing has been done, such a determination may be conveniently made by review of the patient's medical chart.

A mutation is considered to be "associated with obesity" if the mutation is identified in one or more obese patients, and is present at a significantly lower frequency in a non-obese population (as determined by any standard parametric test of statistical significance). MC4R mutations currently known to be associated with obesity include, but are not limited to, frameshift mutations (*e.g.*, deletion of CTCT at codon 211, resulting in a truncated protein, or

insertion of four nucleotides at codon 244), nonsense mutations (*e.g.*, at codon 35, resulting in a truncated protein), and missense mutations (*e.g.*, resulting in amino acid substitution(s) at position 11, 18, 30, 37, 50, 58, 78, 98, 102, 103, 112, 137, 150, 165, 170, 250, 252, 274, 301 and/or 317). The present invention encompasses treatment of patients with any MC4R mutation(s) that are currently known or are subsequently determined to be associated with obesity.

As used herein, a patient is considered "obese" if the patient's body mass index is greater than 28. Body mass index (BMI) may be readily calculated using the following formula:

$$BMI = (\text{weight in kg}) \ / \ (\text{Height in meters})^2$$

The term "MCH receptor" refers to a naturally-occurring mammalian (*e.g.*, human, dog, cat, or monkey) MCH type 1 or type 2 receptor such as the MCH type 1 receptor (MCHR1; *e.g.*, Lakaye et al., *supra*) and the MCH type 2 receptor (MCHR2; An et al., *supra*; Sailer et al., *supra*; Hill et al., *supra*; Mori et al., *supra*). SEQ ID NOs:1 and 2, herein, recite the DNA and amino acid sequences, respectively, of a Cynomolgus macaque MCH1R.

A "MCH receptor antagonist" is a compound that detectably inhibits MCH binding to one or more MCH receptors and/or inhibits MCH receptor-mediated signal transduction, as measured using the representative assays provided in Examples 1 and 2 herein. Antagonists for use within the context of the present invention are generally non-toxic. Within certain embodiments, an MCH receptor antagonist has a relatively low molecular weight (*e.g.*, less than 700 amu) and is multi-aryl (*i.e.*, has a plurality of unfused or fused aryl groups), non-peptide and amino acid free. Such compounds include, but are not limited to, substituted analogues of benzimidazole, 1-benzyl-4-aryl-piperazine, 1-benzyl-4-aryl-piperidine, and phenylcycloalkylmethylamino and phenylalkenylamino compounds. An antagonist binds "specifically" to MCH receptor if it binds to an MCH receptor (total binding minus nonspecific binding) with a K_i that is 10-fold, preferably 100-fold, and more preferably 1000-fold, less than the K_i measured for MCH receptor antagonist binding to other G protein-coupled receptors. An antagonist binds with "high affinity" if the K_i at an MCH receptor is less than 1 micromolar, preferably less than 500 nanomolar, 100 nanomolar or 10 nanomolar. MCH receptor antagonists preferably have minimal agonist activity (*i.e.*, induce an increase in the basal activity of the MCH receptor that is less than 5% of the increase that would be induced by one EC_{50} of MCH), and more preferably have no detectable agonist activity within the assay described in Example 3).

The term "nontoxic" as used herein shall be understood in a relative sense and is intended to refer to any substance that has been approved by the United States Food and Drug Administration ("FDA") for administration to mammals (preferably humans) or, in keeping with

established criteria, is susceptible to approval by the FDA for administration to mammals (preferably humans). Toxicity may also, or alternatively, be evaluated using an assay detecting an effect on cellular ATP production, such as the assay provided in Example 4. Within such an assay, a 100 μM concentration of a non-toxic compound results in ATP levels that are at least 50%, preferably at least 80%, of the ATP levels detected in untreated cells. Other assays that may be used include bacterial reverse mutation assays, such as an Ames test, as well as standard teratogenicity and tumorogenicity assays. Preferably, administration of a compound provided herein at a dose that yields a therapeutically effective *in vivo* concentration does not result in prolongation of heart QT intervals (*i.e.*, as determined by electrocardiography in guinea pigs, minipigs or dogs). When administered daily for five or preferably ten days, such doses also do not cause liver enlargement resulting in an increase of liver to body weight ratio of more than 100%, preferably not more than 75% and more preferably not more than 50% over matched controls in laboratory rodents (*e.g.*, mice or rats). Such doses also preferably do not cause liver enlargement resulting in an increase of liver to body weight ratio of more than 50%, preferably not more than 25%, and more preferably not more than 10% over matched untreated controls in dogs or other non-rodent mammals.

A "prodrug" is a compound that may not be an MCH receptor antagonist, but is modified *in vivo*, following administration to a patient, to produce such an antagonist. For example, a prodrug may be an acylated derivative of an MCH receptor antagonist. Prodrugs include compounds wherein hydroxy, amine or sulfhydryl groups are bonded to any group that, when administered to a mammalian subject, cleaves to form a free hydroxyl, amino or sulfhydryl group, respectively. Examples of prodrugs include, but are not limited to, acetate, formate and benzoate derivatives of alcohol and amine functional groups within an MCH receptor antagonist.

Specific MCH receptor antagonists are generally described herein using standard nomenclature. For compounds having asymmetric centers, it should be understood that (unless otherwise specified) all of the optical isomers and mixtures thereof are encompassed. In addition, compounds with carbon-carbon double bonds may occur in Z- and E- forms, with all isomeric forms of the compounds being included unless otherwise specified. Where a compound exists in various tautomeric forms, a recited compound is not limited to any one specific tautomer, but rather is intended to encompass all tautomeric forms. Certain compounds are described herein using a general formula that includes variables. Unless otherwise specified, each variable within such a formula is defined independently of other variable, and any variable that occurs more than one time in a formula is defined independently at each occurrence. All

MCH receptor antagonists may be present as a free base or as a pharmaceutically acceptable acid addition salt.

As used herein, the term "alkyl" refers to a straight chain, branched chain or cyclic saturated aliphatic hydrocarbon. An alkyl group may be bonded to an atom within a molecule of interest via any chemically suitable portion. Alkyl groups include groups having from 1 to 8 carbon atoms (C_1-C_8alkyl), from 1 to 6 carbon atoms (C_1-C_6alkyl) and from 1 to 4 carbon atoms (C_1-C_4alkyl), such as methyl, ethyl, propyl, isopropyl, n-butyl, sec-butyl, tert-butyl, pentyl, 2-pentyl, isopentyl, neopentyl, hexyl, 2-hexyl, 3-hexyl, 3-methylpentyl, cyclopropyl, cyclopropylmethyl, cyclopentyl, cyclopentylmethyl, cyclohexyl, cycloheptyl and norbornyl. "C_0-C_4alkyl" refers to a bond or a C_1-C_4alkyl group; "C_0-C_8alkyl" refers to a bond or a C_1-C_8alkyl group.

Similarly, "alkenyl" refers to straight or branched chain alkene groups or cycloalkene groups. Within an alkenyl group, one or more unsaturated carbon-carbon double bonds are present. Alkenyl groups include C_2-C_8alkenyl, C_2-C_6alkenyl and C_2-C_4alkenyl groups, which have from 2 to 8, 2 to 6 or 2 to 4 carbon atoms, respectively, such as ethenyl, allyl or isopropenyl. "Alkynyl" refers to straight or branched chain alkyne groups, which have, one or more unsaturated carbon-carbon bonds, at least one of which is a triple bond. Alkynyl groups include C_2-C_8alkynyl, C_2-C_6alkynyl and C_2-C_4alkynyl groups, which have from 2 to 8, 2 to 6 or 2 to 4 carbon atoms, respectively.

By "alkoxy," as used herein, is meant an alkyl, alkenyl or alkynyl group as described above attached via an oxygen bridge. Alkoxy groups include C_1-C_8alkoxy, C_1-C_6alkoxy and C_1-C_4alkoxy groups, which have from 1 to 8, 1 to 6 or 1 to 4 carbon atoms, respectively. Alkoxy groups include, for example, methoxy, ethoxy, propoxy, isopropoxy, n-butoxy, sec-butoxy, tert-butoxy, n-pentoxy, 2-pentoxy, 3-pentoxy, isopentoxy, neopentoxy, hexoxy, 2-hexoxy, 3-hexoxy, and 3-methylpentoxy.

The term "alkanoyl" refers to an acyl group in a linear, branched or cyclic arrangement (*e.g.*, -(C=O)-alkyl). Alkanoyl groups include C_2-C_8alkanoyl, C_2-C_6alkanoyl and C_2-C_4alkanoyl groups, which have from 2 to 8, 2 to 6 or 2 to 4 carbon atoms, respectively.

An "alkanone" is a ketone group in which carbon atoms are in a linear, branched or cyclic alkyl arrangement. "C_3-C_8alkanone," "C_3-C_6alkanone" and "C_3-C_4alkanone" refer to an alkanone having from 3 to 8, 6 or 4 carbon atoms, respectively.

Similarly, "alkyl ether" refers to a linear or branched ether substituent linked via a carbon-carbon bond. Alkyl ether groups include C_2-C_8alkyl ether, C_2-C_6alkyl ether and C_2-C_6alkyl ether groups, which have 2 to 8, 6 or 4 carbon atoms, respectively.

The term "alkoxycarbonyl" refers to an alkoxy group linked via a carbonyl (*e.g.*, a group having the general structure –C(=O)–O–alkyl). Alkoxycarbonyl groups include C_2-C_8, C_2-C_6 and C_2-C_4alkoxycarbonyl groups, which have from 2 to 8, 6 or 4 carbon atoms, respectively.

"C_2-C_6alkylcarboxamido" refers to an alkyl substituent linked via a carboxamide group. In other words, an alkylcarboxamido substituent has the general structure –C(=O)–NH–alkyl.

"C_2-C_6alkylsulfonamido" refers to an alkyl substituent linked via a sulfonamide group. In other words, an alkylcarboxamido substituent has the general structure –SO_2–NH–alkyl.

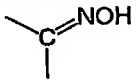
"Alkanoyloxy," as used herein, refers to an alkanoyl group linked via an oxygen bridge (*e.g.*, a group having the general structure –O–C(=O)–alkyl). Alkanoyloxy groups include C_2-C_8, C_2-C_6 and C_2-C_4alkanoyloxy groups, which have from 2 to 8, 6 or 4 carbon atoms, respectively.

The term "C_2-C_6carbonate" refers to an alkoxycarbonyl group linked via an oxygen bridge. In other words, a carbonate group may have the general structure –O–C(=O)–O–alkyl. C_2-C_4carbonate groups contain from 2 to 4 carbon atoms.

The term "C_2-C_6carbamate," as used herein, refers to a group having the general structure –N–C(=O)–O–alkyl. C_2-C_4carbamate groups contain from 2 to 4 carbon atoms.

The term "oxo," as used herein, refers to a keto (C=O) group. An oxo group that is a substituent of a nonaromatic ring results in a conversion of $–CH_2–$ to –C(=O)–. It will be apparent that the introduction of an oxo substituent on an aromatic ring destroys the aromaticity.

The term "oxime" refers to a group of the structure:



If an oxime is designated as a substituent, the carbon atom is generally part of the base structure, with only the =NOH added. The carbon atom of the oxime may, of course, be a member of a carbocyclic or heterocyclic ring. If a ring is aromatic, it will be apparent that the introduction of an oxime substituent destroys the aromaticity.

The term "halogen" includes fluorine, chlorine, bromine and iodine. A "haloalkyl" is a straight chain, branched chain or cyclic alkyl group, substituted with 1 or more halogen atoms (*e.g.*, "haloC_1-C_8alkyl" groups have from 1 to 8 carbon atoms; "haloC_1-C_6alkyl" groups have from 1 to 6 carbon atoms). Examples of haloalkyl groups include, but are not limited to, mono-, di- or tri-fluoromethyl; mono-, di- or tri-chloromethyl; mono-, di-, tri-, tetra- or penta-

fluoroethyl; and mono-, di-, tri-, tetra- or penta-chloroethyl. Typical haloalkyl groups are trifluoromethyl and difluoromethyl. Within certain compounds provided herein, not more than 5 or 3 haloalkyl groups are present. The term "haloalkoxy" refers to a haloalkyl group attached via an oxygen bridge. "HaloC_1-C_6alkoxy" groups have from 1 to 6 carbon atoms.

A dash ("-") that is not between two letters or symbols is used to indicate a point of attachment for a substituent. For example, -$CONH_2$ is attached through the carbon atom.

A "heteroatom" is oxygen, sulfur or nitrogen.

A "substituent," as used herein, refers to a molecular moiety that is covalently bonded to an atom within a molecule of interest. For example, a "ring substituent" may be a moiety such as a halogen, alkyl group, alkoxy group, haloalkyl group or other group as discussed herein that is covalently bonded to an atom (preferably a carbon or nitrogen atom) that is a ring member. The term "substitution" refers to replacing a hydrogen atom in a molecular structure with a substituent as described above, such that the valence on the designated atom is not exceeded, and such that a chemically stable compound (*i.e.*, a compound that can be isolated, characterized, and tested for biological activity) results from the substitution.

Groups that are "optionally substituted" are unsubstituted or are substituted by other than hydrogen at one or more available positions, typically 1, 2, 3, 4 or 5 positions, by one or more suitable groups (which may be the same or different). Such optional substituents include, for example, hydroxy, halogen, cyano, nitro, oxo, oxime, C_1-C_6alkyl, C_2-C_6alkenyl, C_2-C_6alkynyl, C_1-C_6alkoxy, C_2-C_6alkyl ether, C_3-C_6alkanone, C_1-C_6alkylthio, amino, mono- or di-(C_1-C_6alkyl)amino, haloC_1-C_6alkyl, haloC_1-C_6alkoxy, C_2-C_6alkanoyl, C_2-C_6alkoxycarbonyl, C_2-C_6alkanoyloxy, C_2-C_6carbonate, C_2-C_6carbamate, -COOH, -$CONH_2$, mono- or di-(C_1-C_8alkyl)carboxamido, -SO_2NH_2, mono- or di-(C_1-C_6alkyl)amino(C_1-C_6alkyl), mono or di(C_1-C_8alkyl)sulfonamido, carbocyclic groups and heterocyclic groups, as well as any of the foregoing groups further substituted with 1 or more (*e.g.*, from 1 to 5) secondary substituents selected from hydroxy, halogen, cyano, nitro, amino, C_1-C_4alkyl and haloC_1-C_4alkyl. Certain optionally substituted groups are substituted with from 0 to 3 independently selected substituents.

A "carbocycle" or "carbocyclic group" comprises at least one ring formed entirely by carbon-carbon bonds (referred to herein as a carbocyclic ring), and does not contain a heterocyclic ring. Unless otherwise specified, each carbocyclic ring within a carbocycle may be saturated, partially saturated or aromatic. A carbocycle generally has from 1 to 3 fused, pendant or spiro rings; carbocycles within certain embodiments have one ring or two fused rings. Typically, each ring contains from 3 to 8 ring members (*i.e.*, C_3-C_8); C_5-C_7 rings are recited in certain embodiments. Carbocycles comprising fused, pendant or spiro rings typically contain

from 9 to 14 ring members. A "ring member" is a carbon atom or heteroatom that is a part of a ring, and is directly bonded to two other such atoms. A phenyl or pyridyl group, for example, has 6 ring members, regardless of the number of atoms present within ring substituents.

Certain representative carbocycles are optionally substituted cycloalkyl, cycloalkenyl or cycloalkynyl (such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, adamantyl, decahydro-naphthalenyl, octahydro-indenyl, and partially saturated variants of any of the foregoing, such as cyclohexenyl), as well as aromatic groups (*i.e.*, groups that contain at least one aromatic carbocyclic ring, such as phenyl, benzyl, naphthyl, phenoxyl, benzoxyl, phenylethanonyl, fluorenyl, indanyl or 1,2,3,4-tetrahydro-naphthyl). Optional substitutions include those listed above. C_5-C_{10}carbocyclic groups that contain 1 carbocyclic ring or 2 fused carbocyclic rings (for a total of 5 to 10 ring members), optionally substituted as described above, are recited in certain embodiments.

A "heterocycle" or "heterocyclic group" has from 1 to 3 fused, pendant or spiro rings, at least one of which is a heterocyclic ring (*i.e.*, one or more ring atoms is a heteroatom, with the remaining ring atoms being carbon). Typically, a heterocyclic ring comprises 1-4 heteroatoms; within certain embodiments each heterocyclic ring has 1 or 2 heteroatoms per ring. Each heterocyclic ring generally contains from 3 to 8 ring members (rings having from 5 to 7 ring members are recited in certain embodiments), and heterocycles comprising fused, pendant or spiro rings typically contain from 9 to 14 ring members. Heterocycles may be optionally substituted at nitrogen and/or carbon ring members with a variety of substituents, such as those described above. Unless otherwise specified, a heterocycle may be a heterocycloalkyl group (*i.e.*, each ring is saturated or partially saturated) or a heteroaryl group (*i.e.*, at least one ring within the group is aromatic). 3- to 10-membered heterocyclic groups (*i.e.*, groups with from 3 to 10 ring members) that contain 1 heterocyclic ring or 2 fused rings (at least one of which is heterocyclic), optionally substituted as described above, are recited for certain embodiments, as are 5- to 10-membered heterocyclic groups.

Heterocyclic groups include, for example, acridinyl, azepanyl, azocinyl, benzimidazolyl, benzimidazolinyl, benzisothiazolyl, benzisoxazolyl, benzofuranyl, benzothiofuranyl, benzothiophenyl, benzoxazolyl, benzothiazolyl, benzotriazolylcarbazolyl, benztetrazolyl, NH-carbazolyl, carbolinyl, chromanyl, chromenyl, cinnolinyl, decahydroquinolinyl, dihydrofuro[2,3-b]tetrahydrofuran, dihydroisoquinolinyl, dihydrotetrahydrofuranyl, 1,4-dioxa-8-aza-spiro[4.5]dec-8-yl, dithiazinyl, furanyl, furazanyl, imidazolinyl, imidazolidinyl, imidazolyl, indazolyl, indolenyl, indolinyl, indolizinyl, indolyl, isobenzofuranyl, isochromanyl, isoindazolyl, isoindolinyl, isoindolyl, isothiazolyl, isoxazolyl, isoquinolinyl, morpholinyl, naphthyridinyl,

octahydroisoquinolinyl, oxadiazolyl, oxazolidinyl, oxazolyl, phenanthridinyl, phenanthrolinyl, phenazinyl, phenothiazinyl, phenoxathiinyl, phenoxazinyl, phthalazinyl, piperazinyl, piperidinyl, piperidinyl, piperidonyl, pteridinyl, purinyl, pyranyl, pyrazinyl, pyrazolidinyl, pyrazolinyl, pyrazolyl, pyridazinyl, pyridoimidazolyl, pyridooxazolyl, pyridothiazolyl, pyridyl, pyrimidyl, pyrrolidinyl, pyrrolidonyl, pyrrolinyl, pyrrolyl, quinazolinyl, quinolinyl, quinoxalinyl, quinuclidinyl, tetrahydroisoquinolinyl, tetrahydroquinolinyl, tetrazolyl, thiadiazinyl, thiadiazolyl, thianthrenyl, thiazolyl, thienothiazolyl, thienooxazolyl, thienoimidazolyl, thienyl, thiophenyl, thiomorpholinyl and variants thereof in which the sulfur atom is oxidized, triazinyl, xanthenyl and any of the foregoing that are substituted with from 1 to 4 substituents as described above. Within certain embodiments, pyridyl rings are specifically recited.

MELANIN CONCENTRATING HORMONE RECEPTOR ANTAGONISTS

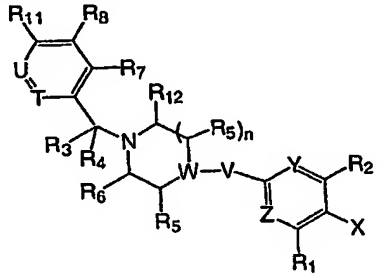
As noted above, the present invention provides compositions and methods useful for the treatment of overeating and obesity, and for reducing body mass index, in patients carrying a MC4R mutation. Compositions provided herein generally comprise a non-toxic melanin concentrating hormone (MCH) receptor antagonist. Such antagonists may be specific for a particular MCH receptor (*e.g.*, type 1 or type 2), or may function at multiple MCH receptors. MCH receptor antagonists for use within the compositions provided herein are, within certain embodiments, low molecular weight (*e.g.*, less than 700 amu), multi-aryl, non-peptide and amino acid free.

As noted above, MCH receptor antagonists for use herein detectably inhibit MCH binding to MCHR1 and/or MCHR2 receptor (as determined using a standard *in vitro* MCH receptor ligand binding assay and/or calcium mobilization assay) at submicromolar concentrations, preferably at nanomolar concentrations, and more preferably at subnanomolar concentrations. References herein to a "MCH receptor ligand binding assay" are intended to refer to the standard *in vitro* receptor binding assay provided in Example 2. Briefly, a competition assay may be performed in which an MCH receptor preparation is incubated with labeled (*e.g.*, ^{125}I) MCH and unlabeled test compound. Within the assays provided herein, the MCH receptor used is preferably a mammalian MCHR1 or MCHR2 receptor, more preferably a human or monkey MCHR1 or MCHR2 receptor. The MCH receptor preparation may be, for example, a membrane preparation from HEK293 cells that recombinantly express a human MCH receptor (*e.g.*, Genbank Accession No. Z86090), monkey MCHR1 receptor (such as the MCHR1 sequence provided in SEQ ID NO:1), or human MCHR1/human beta-2-adrenergic chimeric receptor.

Incubation with an MCH receptor antagonist will result in a decrease in the amount of label bound to the MCH receptor preparation, relative to the amount of label bound in the absence of the compound. Preferably, such a compound exhibits a K_i at an MCH receptor of less than 1 micromolar, binding specifically and with high affinity to an MCH receptor. More preferably, such a compound exhibits a K_i at an MCH receptor of less than 500 nM, 100 nM, 20 nM or 10 nM, within a MCH receptor ligand binding assay performed as described in Example 2.

A representative calcium mobilization assay is provided in Example 3. Generally preferred exhibit EC_{50} values of about 4 micromolar or less, more preferably 1 micromolar or less, still more preferably about 100 nanomolar or less, 10 nanomolar or less or 1 nanomolar or less within a standard *in vitro* MCH receptor mediated calcium mobilization assay, as provided in Example 3.

In certain embodiments, MCH receptor antagonists include substituted 1-benzyl-4-aryl piperazine and piperidine analogues, as described within pending US Application No. 10/126,764 (which is incorporated herein by reference for its teaching of MCH receptor antagonists and the preparation thereof). Briefly, such compounds satisfy Formula I:



Formula I

or a pharmaceutically acceptable salt or hydrate thereof.

Within Formula I, V is a bond or –(C=O)–, and W is nitrogen, CH, COH or CCN. The variable "n" is 1 or 2 (*i.e.*, the heterocyclic ring comprising W may be a 6- or 7-membered ring; within certain embodiments, the ring is a 6-membered ring).

X is selected from halogen, hydroxy, nitro, cyano, -COOH, oxo, and groups of the formula L-M, as defined below. Within certain embodiments, X is halogen, C_1-C_3alkyl, haloC_1-C_3alkyl (*e.g.*, trifluoromethyl), C_1-C_3alkoxy (*e.g.*, methoxy) or haloC_1-C_3alkoxy.

Y and Z are each independently: (i) CH, (ii) nitrogen, or (iii) carbon joined to R_5 to form a carbocyclic or heterocyclic ring comprising W and V having from 5 to 8 ring members. As noted above, any carbocyclic or heterocyclic ring so formed may be saturated, partially saturated or unsaturated, and is optionally substituted with one or more (*e.g.*, from 1 to 3) independently

selected substituents as described above. It will be apparent that such a carbocyclic or heterocyclic ring includes, in addition to W and V, the carbon atom linked to V and a carbon atom adjacent to W. Within certain embodiments, Y and Z are both CH, or one of Y and Z is nitrogen; preferably Y and Z are not both nitrogen. If one of Y or Z is a carbon joined to R_5, the other of Y and Z is either CH or nitrogen.

R_1 and R_2 are each independently selected from: (a) hydrogen, halogen, hydroxy, nitro, cyano, -COOH, and oxo; and (b) groups of the formula L-M, as defined below. Within certain embodiments, R_1 and R_2 are each independently selected from hydrogen, halogen, C_1-C_3alkyl (*e.g.*, methyl), haloC_1-C_3alkyl (*e.g.*, di- or tri-fluoromethyl), C_1-C_3alkoxy and haloC_1-C_3alkoxy. One of R_1 and R_2 is hydrogen in certain embodiments. If R_1 and R_2 are both hydrogen, then V is preferably –(C=O)–.

R_3 is: (i) selected from hydrogen and optionally substituted C_1-C_6alkyl, C_2-C_6alkenyl and haloC_1-C_6alkyl; or (ii) joined to one or both of R_6 and R_{10} to form a carbocyclic or heterocyclic group having one ring or two fused rings, wherein each ring contains from 5 to 8 ring members and 0, 1 or 2 heteroatoms independently chosen from oxygen, nitrogen and sulfur, and wherein each ring is optionally substituted as described above. It will be apparent that any ring formed with R_6 includes the carbon atoms to which R_3 and R_6 are attached, as well as the nitrogen atom linked to these carbon atoms. Similarly, a ring formed with R_{10} includes the carbon atoms to which R_3 and R_{10} are attached, as well as the intervening carbon atom. Certain R_3 groups are hydrogen, C_1-C_3alkyl (*e.g.*, methyl or ethyl), haloC_1-C_3alkyl (*e.g.*, trifluoromethyl) and groups that form a 5-membered, partially saturated ring with R_{10} or a 5- or 6-membered saturated or partially saturated ring with R_6.

R_4 is hydrogen or optionally substituted C_1-C_6alkyl or haloC_1-C_6alkyl; within certain embodiments, R_4 is hydrogen, methyl or trifluoromethyl.

R_5 is: (i) independently selected at each occurrence from hydrogen, halogen, hydroxy, nitro, cyano, amino, oxo, and optionally substituted C_1-C_6alkyl, C_2-C_6alkenyl, C_2-C_6alkynyl, C_1-C_6alkoxy, haloC_1-C_6alkyl, haloC_1-C_6alkoxy, mono- and di(C_1-C_6)alkylamino, and amino(C_1-C_6)alkyl; or (ii) joined to R_6, Y or Z to form a carbocyclic or heterocyclic ring having from 5 to 8 ring members, optionally substituted as described above. For example, R_5 may be a direct bond to R_6, Y or Z, or may be any substituent that, when linked to R_6, Y or Z, results in a carbocyclic or heterocyclic ring of the appropriate size. As noted above, such a carbocyclic or heterocyclic ring includes W and V, as well as the carbon atom linked to V and a carbon atom adjacent to W. Within certain embodiments, each R_5 is independently hydrogen or methyl.

R_6 is: (i) selected from hydrogen, halogen, hydroxy, nitro, cyano, amino, oxo, and optionally substituted C_1-C_6alkyl, C_2-C_6alkenyl, C_2-C_6alkynyl, C_1-C_6alkoxy, haloC_1-C_6alkyl, haloC_1-C_6alkoxy, mono- and di(C_1-C_6)alkylamino, and amino(C_1-C_6)alkyl; or (ii) joined with R_3 or R_5 to form a carbocyclic or heterocyclic group as described above. Within certain embodiments, R_6 is hydrogen or methyl, or is joined with R_3 to form a saturated or partially saturated 5- or 6-membered ring.

R_7 is: (a) selected from hydrogen, halogen, hydroxy, nitro, cyano, -COOH, oxo and groups of the formula L-M, as defined below; or (b) joined with R_8 or R_{12} to form a fused 5- or 6-membered carbocyclic or heterocyclic group (saturated, partially saturated or unsaturated, and optionally substituted). It will be apparent that any ring formed with R_8 includes the carbon atoms to which R_7 and R_8 are attached. Similarly, a ring formed with R_{12} includes the carbon atoms to which R_7 and R_{12} are attached. Within certain embodiments, R_7 is hydrogen.

R_8 is: (a) selected from hydrogen, halogen, hydroxy, nitro, cyano, -COOH, oxo and groups of the formula L-M, as defined below; or (b) joined with R_7 or R_{11} to form a fused 5- to 10-member carbocyclic or heterocyclic group. Within certain embodiments, R_8 is hydrogen, halogen, C_1-C_3alkyl, haloC_1-C_3alkyl, C_1-C_3alkoxy or haloC_1-C_3alkoxy, or R_8 is joined with R_7 or R_{11} to form a fused 5- or 6-membered ring, or a fused 9- or 10-membered bicyclic group.

U is N, O or CR_9; and R_9 is (a) selected from hydrogen, halogen, hydroxy, nitro, cyano, COOH, oxo, and groups of the formula L-M, as defined below; or (b) joined with R_{10} or R_{11} to form a fused 5- to 10-member carbocyclic or heterocyclic group. It will be apparent that any ring formed with R_{10} includes the carbon atoms to which R_9 and R_{10} are attached. Similarly, a ring formed with R_{11} includes the carbon atoms to which R_9 and R_{11} are attached. In certain embodiments, R_9 is hydrogen, halogen, C_1-C_3alkyl (*e.g.*, methyl) or C_1-C_3alkoxy (*e.g.*, methoxy), or is fused with R_{10} or R_{11} to form a 6-membered aromatic ring.

T is N, O or CR_{10}; and R_{10} is: (a) selected from hydrogen, halogen, hydroxy, nitro, cyano, -COOH, oxo and groups of the formula L-M, as defined below; or (b) joined with R_3, R_8 or R_9 to form a carbocyclic or heterocyclic group as described above.

R_{11} is: (a) selected from hydrogen, halogen, hydroxy, nitro, cyano, -COOH, oxo and groups of the formula L-M, as defined below; or (b) joined with one or both of R_8 and R_9 to form a fused 5- to 10-member carbocyclic or heterocyclic group. Within certain embodiments, R_{11} is hydrogen, hydroxy, halogen, C_1-C_3alkyl, haloC_1-C_3alkyl (*e.g.*, trifluoromethyl) or C_1-C_3alkoxy (*e.g.*, methoxy or ethoxy).

R_{12} is: (i) independently selected at each occurrence from hydrogen, halogen, hydroxy, nitro, cyano, amino, oxo, and optionally substituted C_1-C_6alkyl, C_2-C_6alkenyl, C_2-C_6alkynyl, C_1-

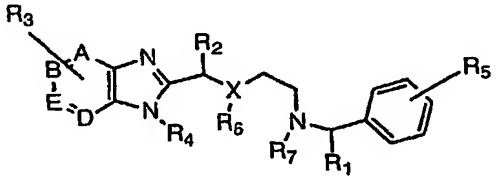
C_6alkoxy, haloC_1-C_6alkyl, haloC_1-C_6alkoxy, mono- and di(C_1-C_6)alkylamino, and amino(C_1-C_6)alkyl; or (ii) joined to R_7 to form a fused carbocyclic or heterocyclic ring as described above. Preferably R_{12} is hydrogen or methyl.

L is independently at each occurrence a bond, $-NR_{13}$- –O-, $-SO_2$-, -SO_2NH-, C(=O)NR_{13}- or NR_{13}C(=O)-, wherein R_{13} is independently selected at each occurrence from hydrogen, C_1-C_6alkyl, C_2-C_6alkenyl, C_2-C_6alkynyl and haloC_1-C_6alkyl.

M is independently at each occurrence hydrogen or optionally substituted C_1-C_6alkyl, C_2-C_6alkenyl, C_2-C_6alkynyl, haloC_1-C_6alkyl, amino(C_1-C_6)alkyl or a 5- to 10-membered carbocycle. As noted above, any carbocycle may be saturated, partially saturated or unsaturated, and may (but need not) be substituted with one or more (*e.g.*, from 1 to 3) substituents.

Within certain embodiments, at least one of R_{10}, R_3 and R_4 is not hydrogen. In particular, certain compounds of Formula I in which R_{11} is halogen and V is a bond do not contain a hydrogen at all three of the positions designated R_{10}, R_3 and R_4.

Within other embodiments, MCH receptor antagonists for use within the present compositions are substituted benzimidazole analogues as described within pending U.S. Provisional Application No. 60/347,573, filed January 10, 2002 (which is incorporated herein by reference for its teaching of MCH receptor antagonists and the preparation thereof). Such antagonists generally satisfy Formula II:



Formula II

or a pharmaceutically acceptable salt or hydrate thereof.

Within Formula II, A, B, E, D and X each independently represent CH or N, with the proviso that not more than two of A, B, E and D represent N.

R_1 is: (i) hydrogen, -C(=O)-NH_2, -SO_2NH_2 or -COOH; or (ii) C_1-C_8alkyl, C_2-C_8alkenyl, C_2-C_8alkynyl, C_1-C_8alkoxy, C_2-C_8alkanoyl, C_2-C_8alkyl ether, C_2-C_8alkanoyloxy, C_2-C_8alkoxycarbonyl, C_2-C_8carbonate, C_1-C_8alkylthio, mono- or diC_1-C_8alkylamino, C_2-C_8carbamate, mono or di(C_1-C_8alkyl)sulfonamido, or mono or di(C_1-C_8alkyl)carboxamido, each of which is optionally substituted with from 1 to 9 substituents independently selected from hydroxy, halogen, amino, cyano, nitro, C_1-C_8alkyl and haloC_1-C_8)alkyl.

R_2 is: (i) hydrogen, halogen, hydroxy, -C(=O)-NH_2, -SO_2NH_2 or -COOH; or (ii) C_1-C_8alkyl, C_2-C_8alkenyl, C_2-C_8alkynyl, C_1-C_8alkoxy, C_2-C_8alkanoyl, C_2-C_8alkyl ether, C_2-

C_8alkanoyloxy, C_1-C_8alkoxycarbonyl, C_2-C_8carbonate, C_1-C_8alkylthio, mono- or di(C_1-C_8alkyl)amino, C_2-C_8carbamate, mono or di(C_1-C_8alkyl)sulfonamido or mono- or di(C_1-C_8alkyl)carboxamido, each of which is optionally substituted with from 1 to 9 (*e.g.*, from 1 to 3) substituents independently selected from hydroxy, halogen, amino, cyano, nitro, C_1-C_8alkyl and haloC_1-C_8alkyl.

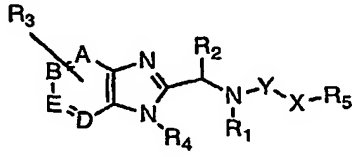
R_3 represents 0 to 4 substituents, each of which is linked to a carbon atom at A, B, E or D and is independently selected from: (i) halogen, hydroxy, amino, cyano, nitro, -C(=O)-NH_2, -SO_2NH_2 and -COOH; and (ii) C_1-C_8alkyl, C_2-C_8alkenyl, C_2-C_8alkynyl, C_1-C_8alkoxy, C_2-C_8alkanoyl, C_2-C_8alkanoyloxy, C_2-C_8alkoxycarbonyl, C_2-C_8carbonate, C_1-C_8alkylthio, mono- and di(C_1-C_8alkyl)amino, C_2-C_8carbamate, mono and di(C_1-C_8alkyl)sulfonamido, and mono and di(C_1-C_8alkyl)carboxamido, each of which is optionally substituted with from 1 to 9 secondary substituents independently selected from hydroxy, halogen, amino, cyano, nitro, C_1-C_8alkyl and haloC_1-C_8alkyl. Within certain embodiments, R_3 substituents are absent or selected from trifluoromethyl and cyano.

R_4 is: (i) hydrogen, -C(=O)-NH_2, -SO_2NH_2 or -COOH; (ii) C_1-C_8alkyl, C_2-C_8alkenyl, C_2-C_8alkynyl, C_1-C_8alkoxy, C_2-C_8alkanoyl, C_2-C_8alkanoyloxy, C_2-C_8alkoxycarbonyl, C_2-C_8carbonate, C_1-C_8alkylthio, mono- and di(C_1-C_8alkyl)amino, C_2-C_8carbamate, mono and di(C_1-C_8alkyl)sulfonamido, and mono and di(C_1-C_8alkyl)carboxamido, each of which is optionally substituted with from 1 to 9 secondary substituents independently selected from hydroxy, halogen, amino, cyano, nitro, C_1-C_8alkyl and haloC_1-C_8alkyl; or (iii) L-R_A, wherein L is C_1-C_6alkyl; and R_A is a 5- to 7-membered carbocyclic or heterocyclic ring, optionally substituted with from 1 to 4 substituents independently selected from hydroxy, halogen, amino, cyano, nitro, C_1-C_8alkyl, haloC_1-C_8alkyl, C_1-C_8alkoxy, haloC_1-C_8alkoxy, C_2-C_8alkanoyl, C_2-C_8alkanoyloxy, C_2-C_8alkoxycarbonyl, C_2-C_8carbonate, C_1-C_8alkylthio, mono- and di(C_1-C_8alkyl)amino, C_2-C_8carbamate and 5- to 10-member carbocyclic and heterocyclic groups.

R_5 represents from 0 to 3 substituents, each of which is independently selected from: (i) halogen, hydroxy, amino, cyano, nitro, -C(=O)-NH_2, -SO_2NH_2 and -COOH; or (ii) C_1-C_8alkyl, C_2-C_8alkenyl, C_2-C_8alkynyl, C_1-C_8alkoxy, C_2-C_8alkanoyl, C_2-C_8alkanoyloxy, C_2-C_8alkoxycarbonyl, C_2-C_8carbonate, C_1-C_8alkylthio, mono- and di(C_1-C_8alkyl)amino, C_2-C_8carbamate, mono and di(C_1-C_8alkyl)sulfonamido, mono and di(C_1-C_8alkyl)carboxamido, and fused and pendant 3- to 10-member carbocyclic and heterocyclic groups, each of which is optionally substituted with from 1 to 9 secondary substituents independently selected from hydroxy, halogen, amino, cyano, nitro, C_1-C_8alkyl and haloC_1-C_8alkyl.

R_6 and R_7 are each independently: (i) hydrogen, amino, cyano, nitro, -C(=O)-NH_2, -SO_2NH_2 or -COOH; (ii) C_1-C_8alkyl, C_2-C_8alkenyl, C_2-C_8alkynyl, C_1-C_8alkoxy, C_2-C_8alkanoyl, C_2-C_8alkyl ether, C_2-C_8alkanoyloxy, C_2-C_8alkoxycarbonyl, C_2-C_8carbonate, C_1-C_8alkylthio, mono- or di(C_1-C_8alkyl)amino, C_2-C_8carbamate, mono or di(C_1-C_8alkyl)sulfonamido, or mono or di(C_1-C_8alkyl)carboxamido, each of which is optionally substituted with from 1 to 9 substituents independently selected from hydroxy, halogen, amino, cyano, nitro, C_1-C_8alkyl and haloC_1-C_8alkyl; or (iii) joined to form, with XCH_2CH_2N, a 5- to 7-membered heterocyclic ring that is optionally substituted with from 1 to 3 substituents independently selected from hydroxy, halogen, amino, cyano, nitro, C_1-C_8alkyl and haloC_1-C_8alkyl.

Within certain embodiments, compounds for use within the present invention are as described within pending US Provisional Application No. 60/347,279, filed January 10, 2002 (which is incorporated herein by reference for its teaching of MCH receptor antagonists and the preparation thereof). Such compounds generally satisfy Formula III:



Formula III

or a pharmaceutically acceptable salt or hydrate thereof.

Within Formula III, A, B, E, D and R_3 are as described for Formula II.

R_1 of Formula III is generally a small nonaromatic group, selected from hydrogen, -C(=O)NH_2, -SO_2NH_2, -COOH, C_1-C_8alkyl, C_2-C_8alkenyl, C_2-C_8alkynyl, C_2-C_8alkanoyl, C_2-C_8alkyl ether, C_1-C_8alkylthio, mono- and di(C_1-C_8alkyl)amino, mono and di(C_1-C_8alkyl)sulfonamido and mono and di(C_1-C_8alkyl)carboxamido. Such R_1 groups may, but need not, be substituted with from 1 to 9 (*e.g.*, from 1 to 3) substituents independently selected from hydroxy, halogen, amino, cyano, nitro, C_1-C_8alkyl and haloC_1-C_8alkyl and mono- and di(C_1-C_8alkyl)amino. Alternatively, R_1 is joined with R_2 to form a 5- to 7-member heterocyclic ring that comprises the nitrogen to which R_1 is bound and the carbon to which R_2 is bound, optionally substituted with from 1 to 3 substituents independently selected from hydroxy, halogen, amino, cyano, nitro, C_1-C_8alkyl and haloC_1-C_8alkyl. Within certain embodiments, R_1 is hydrogen or a straight or branched chain lower alkyl or cycloalkyl with 1 to 6 carbon atoms (such as methyl, ethyl, isoamyl, isobutyl, n-butyl, n-propyl, cyclopropylmethyl or cyclopentylmethyl), a C_2-C_6alkyl ether or di(C_1-C_6alkyl)amino(C_1-C_6alkyl).

R_2 of Formula III is also a small, typically nonaromatic group, and is selected from hydrogen, -C(=O)NH_2, -SO_2NH_2, –COOH, C_1-C_8alkyl, C_2-C_8alkenyl, C_2-C_8alkynyl, C_1-

C_8alkoxy, C_2-C_8alkanoyl, C_2-C_8alkyl ether, C_2-C_8alkanoyloxy, C_2-C_8alkoxycarbonyl, C_2-C_8carbonate, C_1-C_8alkylthio, mono- and di(C_1-C_8alkyl)amino, C_2-C_8carbamate, mono and di(C_1-C_8alkyl)sulfonamido and mono- and di(C_1-C_8alkyl)carboxamido. Such R_2 groups may, but need not, be substituted with from 1 to 9 (*e.g.*, from 1 to 3) substituents independently selected from hydroxy, halogen, amino, cyano, nitro, C_1-C_8alkyl and haloC_1-C_8alkyl. Alternatively, R_2 is joined with R_1 to form a 5- to 7-member heterocyclic ring as described above. Within certain embodiments, R_2 is hydrogen or a straight or branched chain lower alkyl or cycloalkyl with 1 to 6 carbon atoms (such as methyl, ethyl, isoamyl, isobutyl, n-butyl, n-propyl or cyclopropylmethyl).

R_4 is a group that comprises (i) an aromatic ring or an alkenyl group, linked to (ii) a tertiary amine. Within certain embodiments, R_4 is L-R_A-Q-M, wherein L is C_1-C_4alkyl, (*e.g.*, C_1-C_3alkyl or $-CH_2-$); R_A is phenyl, optionally substituted with from 1 to 3 substituents independently selected from hydroxy, halogen, amino, cyano, nitro, C_1-C_6alkyl and haloC_1-C_6alkyl; Q is a molecular unit selected from C_0-C_3alkyl, C_2-C_3alkenyl, C_2-C_3alkynyl, C_0-C_3alkoxy and C_0-C_3alkylthio, linked to R_A at position 2; and M is tertiary amine. Within other embodiments, R_4 is L-R_B-Q-M, wherein L and M are as defined above; R_B is C_2-C_6alkenyl, optionally substituted with from 1 to 3 substituents independently selected from hydroxy, halogen, amino, cyano, nitro, C_1-C_6alkyl and haloC_1-C_6alkyl; and Q is a molecular unit selected from C_0-C_3alkyl, C_2-C_3alkenyl, C_2-C_3alkynyl, C_0-C_3alkoxy and C_0-C_3alkylthio.

Y, within Formula III, may be $-CH_2-$, -(C=O)-, -C(=S)-, -S(=O)- or $-(SO_2)-$.

X, within Formula III, represents C_0-C_3alkyl, C_2-C_3alkenyl, C_2-C_3alkynyl, C_0-C_3alkoxy, C_2-C_6alkoxycarbonyl or C_0-C_3alkylthio.

R_5 of Formula III represents a carbocyclic or heterocyclic group having from 1 to 3 fused or pendant rings, at least one of which is aromatic. Each ring within R_5 contains from 5 to 8 ring members, and is optionally substituted by one or more substituents that are independently selected from: hydrogen, halogen, cyano, nitro, C_1-C_8alkyl, C_2-C_8alkenyl, C_2-C_8alkynyl, C_1-C_8alkoxy, C_1-C_8alkylthio, hydroxy, amino, mono and di(C_1-C_8alkyl)amino (*e.g.*, C_3-C_7cycloalkylamino), (C_3-C_7cycloalkyl)C_0-C_3alkyl, haloC_1-C_8alkyl, haloC_1-C_8alkoxy, C_2-C_8alkanoyl, C_2-C_8alkoxycarbonyl, -COOH, $-CONH_2$, mono- and di-(C_1-C_8alkyl)carboxamido,-SO_2NH_2, and mono and di(C_1-C_8alkyl)sulfonamido. A ring within R_5 may be directly linked to X, or may be linked via a ring substituent.

Within further embodiments, compounds for use within the present invention are as described within PCT International Application No. US01/41289, published as WO 02/04433 on January 17, 2002, and hereby incorporated by reference for its teaching of

phenylcycloalkylmethylamino and phenylalkenylamino MCH receptor antagonists and the preparation thereof.

It will be apparent that the specific compounds recited above are illustrative examples of MCH receptor antagonists, and are not intended to limit the scope of the present invention.

As noted above, compositions of the present invention may encompass a pharmaceutically acceptable salt of a MCH receptor antagonist. As used herein, a "pharmaceutically acceptable salt" is an acid or base salt that is generally considered in the art to be suitable for use in contact with the tissues of human beings or animals without excessive toxicity, irritation, allergic response, or other problem or complication. Such salts include mineral and organic acid salts of basic residues such as amines, as well as alkali or organic salts of acidic residues such as carboxylic acids. Specific pharmaceutical salts include, but are not limited to, salts of acids such as hydrochloric, phosphoric, hydrobromic, malic, glycolic, fumaric, sulfuric, sulfamic, sulfanilic, formic, toluenesulfonic, methanesulfonic, ethane disulfonic, 2-hydroxyethylsulfonic, nitric, benzoic, 2-acetoxybenzoic, citric, tartaric, lactic, stearic, salicylic, glutamic, ascorbic, pamoic, succinic, fumaric, maleic, propionic, hydroxymaleic, hydroiodic, phenylacetic, alkanoic such as acetic, HOOC-$(CH_2)_n$-COOH where n is 0-4, and the like. Similarly, pharmaceutically acceptable cations include, but are not limited to sodium, potassium, calcium, aluminum, lithium and ammonium. Those of ordinary skill in the art will recognize further pharmaceutically acceptable salts, including those listed by *Remington's Pharmaceutical Sciences*, 17th ed., Mack Publishing Company, Easton, PA, p. 1418 (1985). Accordingly, the present disclosure should be construed to include all pharmaceutically acceptable salts of MCH receptor antagonists.

A wide variety of synthetic procedures are available for the preparation of pharmaceutically acceptable salts. In general, a pharmaceutically acceptable salt can be synthesized from a parent compound that contains a basic or acidic moiety by any conventional chemical method. Briefly, such salts can be prepared by reacting the free acid or base form of these compounds with a stoichiometric amount of the appropriate base or acid in water or in an organic solvent, or in a mixture of the two; generally, nonaqueous media like ether, ethyl acetate, ethanol, isopropanol, or acetonitrile are preferred.

Prodrugs of MCH receptor antagonists may be prepared by modifying functional groups present in the compounds in such a way that the modifications are cleaved to the parent compounds. Prodrugs include compounds wherein hydroxy, amine or sulfhydryl groups are bonded to any group that, when administered to a mammalian subject, cleaves to form a free hydroxyl, amino, or sulfhydryl group, respectively. Examples of prodrugs include, but are not

limited to, acetate, formate and benzoate derivatives of alcohol and amine functional groups within an MCH receptor antagonist. Preferred prodrugs include acylated derivatives. Those of ordinary skill in the art will recognize various synthetic methods that may be employed to prepare prodrugs of an MCH receptor antagonist.

PHARMACEUTICAL COMPOSITIONS

The practice of the present invention employs pharmaceutical compositions comprising a MCH receptor antagonist, together with at least one physiologically acceptable carrier or excipient. Pharmaceutical compositions may comprise, for example, water, buffers (*e.g.*, neutral buffered saline or phosphate buffered saline), ethanol, mineral oil, vegetable oil, dimethylsulfoxide, carbohydrates (*e.g.*, glucose, mannose, sucrose or dextrans), mannitol, proteins, adjuvants, polypeptides or amino acids such as glycine, antioxidants, chelating agents such as EDTA or glutathione and/or preservatives. Certain pharmaceutical compositions are formulated for oral delivery to humans or other animals (*e.g.*, companion animals such as dogs).

If desired, other active ingredients may also be included, such as leptin, a leptin receptor agonist, a melanocortin receptor 4 (MC4) agonist, sibutramine, dexenfluramine, a growth hormone secretagogue, a beta-3 agonist, a 5HT-2 agonist, an orexin antagonist, a neuropeptide Y_1 or Y_5 antagonist, a galanin antagonist, a CCK agonist, a GLP-1 agonist and/or a corticotropin-releasing hormone agonist.

Pharmaceutical compositions may be formulated for any appropriate manner of administration, including, for example, topical, oral, nasal, rectal or parenteral administration. The term parenteral as used herein includes subcutaneous, intradermal, intravascular (*e.g.*, intravenous), intramuscular, spinal, intracranial, intrathecal and intraperitoneal injection, as well as any similar injection or infusion technique. In certain embodiments, compositions in a form suitable for oral use are preferred. Such forms include, for example, tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsion, hard or soft capsules, or syrups or elixirs. Within yet other embodiments, compositions of the present invention may be formulated as a lyophilizate.

Compositions intended for oral use may further comprise one or more components such as sweetening agents, flavoring agents, coloring agents and preserving agents in order to provide appealing and palatable preparations. Tablets contain the active ingredient in admixture with physiologically acceptable excipients that are suitable for the manufacture of tablets. Such excipients include, for example, inert diluents (*e.g.*, calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate), granulating and disintegrating agents (*e.g.*,

corn starch or alginic acid), binding agents (*e.g.*, starch, gelatin or acacia) and lubricating agents (*e.g.*, magnesium stearate, stearic acid or talc). The tablets may be uncoated or they may be coated by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monosterate or glyceryl distearate may be employed.

Formulations for oral use may also be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent (*e.g.*, calcium carbonate, calcium phosphate or kaolin), or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium (*e.g.*, peanut oil, liquid paraffin or olive oil).

Aqueous suspensions comprise the active materials in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients are suspending agents (*e.g.*, sodium carboxymethylcellulose, methylcellulose, hydropropylmethylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia); and dispersing or wetting agents (*e.g.*, naturally-occurring phosphatides such as lecithin, condensation products of an alkylene oxide with fatty acids such as polyoxyethylene stearate, condensation products of ethylene oxide with long chain aliphatic alcohols such as heptadecaethyleneoxycetanol, condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides such as polyethylene sorbitan monooleate). Aqueous suspensions may also contain one or more preservatives, for example ethyl, or n-propyl p-hydroxybenzoate, one or more coloring agents, one or more flavoring agents, and one or more sweetening agents, such as sucrose or saccharin.

Oily suspensions may be formulated by suspending the active ingredients in a vegetable oil (*e.g.*, arachis oil, olive oil, sesame oil or coconut oil) or in a mineral oil such as liquid paraffin. The oily suspensions may contain a thickening agent such as beeswax, hard paraffin or cetyl alcohol. Sweetening agents such as those set forth above, and/or flavoring agents may be added to provide palatable oral preparations. Such suspension may be preserved by the addition of an anti-oxidant such as ascorbic acid.

Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those already mentioned above. Additional excipients, for example sweetening, flavoring and coloring agents, may also be present.

Pharmaceutical compositions may also be in the form of oil-in-water emulsions. The oily phase may be a vegetable oil (*e.g.*, olive oil or arachis oil) or a mineral oil (*e.g.*, liquid paraffin) or mixtures thereof. Suitable emulsifying agents may be naturally-occurring gums (*e.g.*, gum acacia or gum tragacanth), naturally-occurring phosphatides (*e.g.*, soy bean, lecithin, and esters or partial esters derived from fatty acids and hexitol), anhydrides (*e.g.*, sorbitan monoleate) and condensation products of partial esters derived from fatty acids and hexitol with ethylene oxide (*e.g.*, polyoxyethylene sorbitan monoleate). The emulsions may also contain sweetening and/or flavoring agents.

Syrups and elixirs may be formulated with sweetening agents, such as glycerol, propylene glycol, sorbitol or sucrose. Such formulations may also comprise one or more demulcents, preservatives, flavoring agents and/or coloring agents.

A pharmaceutical composition may be prepared as a sterile injectible aqueous or oleaginous suspension. The MCH receptor antagonist, depending on the vehicle and concentration used, can either be suspended or dissolved in the vehicle. Such a composition may be formulated according to the known art using suitable dispersing, wetting agents and/or suspending agents such as those mentioned above. Among the acceptable vehicles and solvents that may be employed are water, 1,3-butanediol, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils may be employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed, including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectible compositions, and adjuvants such as local anesthetics, preservatives and/or buffering agents can be dissolved in the vehicle.

Compositions may also be prepared in the form of suppositories (*e.g.*, for rectal administration). Such compositions can be prepared by mixing the drug with a suitable non-irritating excipient that is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum to release the drug. Suitable excipients include, for example, cocoa butter and polyethylene glycols.

For administration to non-human animals, the composition may also be added to animal feed or drinking water. It may be convenient to formulate animal feed and drinking water compositions so that the animal takes in an appropriate quantity of the composition along with its diet. It may also be convenient to present the composition as a premix for addition to feed or drinking water.

Pharmaceutical compositions may be formulated as sustained release formulations (*i.e.*, a formulation such as a capsule that effects a slow release of MCH receptor antagonist following

administration). Such formulations may generally be prepared using well known technology and administered by, for example, oral, rectal or subcutaneous implantation, or by implantation at the desired target site. Carriers for use within such formulations are biocompatible, and may also be biodegradable; preferably the formulation provides a relatively constant level of MCH receptor antagonist release. The amount of antagonist contained within a sustained release formulation depends upon the site of implantation, the rate and expected duration of release and the nature of the condition to be treated or prevented.

MCH receptor antagonists are generally present within a pharmaceutical composition in a therapeutically effective amount. A therapeutically effective amount is an amount that results in a discernible patient benefit, such as decreased BMI, decreased food intake and/or weight loss, following repeated administration (*e.g.*, from 1 to 4 times per day for a period of weeks or months). A preferred concentration is one sufficient to inhibit the binding of MCH to MCHR1 receptor *in vitro*. Compositions providing dosage levels ranging from about 0.1 mg to about 140 mg per kilogram of body weight per day are preferred (about 0.5 mg to about 7 g per human patient per day). The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. Dosage unit forms will generally contain between from about 1 mg to about 500 mg of an active ingredient. It will be understood, however, that the optimal dose for any particular patient will depend upon a variety of factors, including the activity of the specific compound employed; the age, body weight, general health, sex and diet of the patient; the time and route of administration; the rate of excretion; any simultaneous treatment, such as a drug combination; and the type and severity of the particular disease undergoing treatment. Optimal dosages may be established using routine testing, and procedures that are well known in the art.

METHODS OF USE

The present invention provides methods for treating health conditions associated with MC4 receptor mutations, such as obesity and overeating, and for reducing the body mass index of a patient carrying a MC4R mutation. Obesity and overeating may be diagnosed and monitored using criteria that have been established in the art. Patients may include humans, domesticated companion animals (pets, such as dogs) and livestock animals, and are typically obese at the time of initiating treatment.

In general, prior to treatment, a determination is made as to whether or not the patient carries a MC4R mutation, as defined above. Such mutations include, but are not limited to,

deletion of CTCT at codon 211, insertion of four nucleotides at codon 244, nonsense mutation at position 35, and missense mutations (*e.g.*, Thr11Ser, Arg18Cys, Ser30Phe, Asp37Val, Val50Met, Ser58Cys, Pro78Leu, Gly98Arg, Ile102Ser, Val103Ile, Thr112Met, Ile137Thr, Thr150Ile, Arg165Trp, Ile170Val, Leu250Gln, Gly252Ser, Asn274Ser, Ile301Thr and/or Ile317Thr).

A determination as to whether or not the patient carries a MC4R mutation may be made by review of the patient's chart, or using standard diagnostic methods. As an initial screen, a patient may (but need not) be evaluated for characteristics commonly associated with MC4R mutations, including early onset obesity associated with hyperphagia, tall stature, high blood pressure, hyperinsulinemia in the absence of diabetes and preserved reproductive function. The presence of a MC4R mutation may be determined, for example, via PCR assay, in which a MC4R nucleotide sequence in a sample (*e.g.*, tissue or body fluid) obtained from a patient is compared to a reference MC4R sequence for the patient's species. Suitable PCR assays will be apparent to those of ordinary skill in the art and include, for example, assays described by Hinney et al. (1999) *J. Clin. Endocrinology and Metabolism 84*:1483-86 and Vaisse et al. (2000) *J. Clin. Invest 106*:253-62. It will be apparent that this determination does not require a comparison of the complete MC4R sequences. Rather, the determination may be made by simply assaying the patient's MC4R nucleotide sequence(s) for the presence of a specific nucleotide or series of nucleotides that are associated with obesity.

If a patient carries such a mutation, treatment involves administering a non-toxic melanin concentrating hormone (MCH) receptor antagonist to the patient. The amount administered is generally an amount that is effective to reduce the body mass index of the patient upon repeated administration. In other words, the amount in one dose need not have a detectable effect on body mass index; however, when administered repeatedly as described herein, the amount should be sufficient to detectably reduce body mass index.

Frequency of dosage may vary depending on the compound used and the particular condition to be treated. In general, a dosage regimen of 4 times daily or less is preferred, with 1 or 2 times daily particularly preferred. The specific dose for any particular patient will depend upon a variety of factors discussed above. In general, the use of the minimum dosage that is sufficient to provide effective therapy is preferred. Patients may generally be monitored for therapeutic effectiveness using assays suitable for the condition being treated or prevented, which will be familiar to those of ordinary skill in the art. For example, treatment is considered to be effective if it results in a statistically significant decrease in weight, BMI or food intake.

The following Examples are offered by way of illustration and not by way of limitation. Unless otherwise specified all reagents and solvents are of standard commercial grade and are used without further purification.

Examples

Example 1

Effect of MCH Receptor Antagonist on Food Consumption Stimulated by Reduced MC4 Receptor Activity

This Example illustrates an *in vivo* assay for use in confirming the ability of a MCH receptor antagonist to inhibit excess food consumption resulting from decreased MC4 activity.

Experimentally naïve male Sprague Dawley rats (Sasco, St. Louis, MO) weighing between 250 and 300 grams are housed in stainless steel hanging cages in a temperature and humidity controlled animal facility (22±2°C, 40-70% relative humidity) with a 12 hour light/dark cycle. Rats are implanted with a 26 g stainless steel cannula aimed at the lateral ventricle. After one week of recovery, 5, 10 or 20 mg/kg MCH receptor antagonist is administered orally in 2% d-α-tocopherol polyethylene glycol succinate to test animals (with vehicle alone administered to control animals) 30 minutes before ICV administration of 6 nmol HS014 (Phoenix Peptide (Belmont, CA); dissolved in distilled water) or distilled water vehicle in a volume of 5 μL. Rats are then placed in their home cages and allowed free access to pre-weighed Purina chow pellets and water. Food consumption is measured 2 hours post ICV injection.

A one-way ANOVA is conducted on the food consumption measurements. Significant dose effects ($p<0.05$) are further analyzed using a Fisher LSD test. Animals that receive HS014 (a cyclic analogue of MSH that functions as a selective MC4 receptor antagonist) eat significantly more food than animals that receive an ICV injection of water vehicle ($p<0.05$). Animals administered HS014 and MCH receptor antagonist eat significantly less than animals that receive HS014 alone ($p<0.05$). Preferably, the level of food consumption in animals treated with HS014 and 20 mg/kg MCH receptor antagonist is not significantly different from the level of consumption in animals treated with vehicle alone (*i.e.*, without HS014).

Example 2

Melanin Concentrating Hormone Receptor Binding Assay

This Example illustrates a standard assay of melanin concentrating hormone receptor binding that may be used to determine the binding affinity of compounds for the MCH receptor.

Total RNA was prepared from Cynomolgus macaque hypothalamus. Monkey hypothalamic cDNA was prepared using random primers and reverse transcriptase according to standard methods. A cDNA encoding the monkey MCH1 receptor was obtained via PCR amplification using the forward (5') Primer of SEQ ID NO:3 and the reverse (3') Primer of SEQ ID NO:4. The full length PCR product was initially cloned into the vector pCR 2.1 (Invitrogen, Carlsbad, CA). The cDNA was reamplified using a forward primer engineered to include an optimal translation initiation site (Kozak sequence). A cDNA expression cassette fragment encoding the monkey MCH1 receptor was blunt end ligated into the PCR-SCRIPT vector (STRATAGENE, La Jolla, CA). The receptor sequence was excised from this vector using EcoRI and Not I and subcloned into the EcoRI/Not site of PCDNA3.1 (INVITROGEN Corp., Carlsbad, CA). The MCH1 receptor DNA sequence is provided in SEQ ID NO:1, with the encoded amino acid sequence provided in SEQ ID NO:2.

HEK 293 cells (American Type Culture Collection, Manassas, VA) were stably transfected with the MCH receptor expression vector via standard calcium phosphate precipitation, and were grown to confluency (approximately 48-72 hours) in DMEM high glucose culture medium (catalog #10-017-CV, MEDIATECH, Herndon, VA) supplemented with 10% fetal bovine serum and 25 mM HEPES, and 500 µg/ml G418, for 48-72 hours at 37°C, 5% CO_2. The cells were pelleted by gentle centrifugation. Cell pellets were washed twice with cold PBS, harvested in cold PBS containing 5 mM EDTA, and stored at –80°C.

At the time of assay, pellets were thawed by addition of wash buffer (25 mM Hepes with 1.0 mM $CaCl_2$, 5.0 mM $MgCl_2$, 120 mM NaCl, PH7.4) and homogenized for 30 seconds using a BRINKMAN POLYTRON, setting 5. Cells were centrifuged for 10 minutes at 48,000 x g. The supernatant was discarded and the pellet was resuspended in fresh wash buffer, and homogenized again. An aliquot of this membrane homogenate was used to determine protein concentration via the Bradford method (BIO-RAD Protein Assay Kit, #500-0001, BIO-RAD, Hercules, CA). By this measure, a 1-liter culture of cells typically yields 50-75 mg of total membrane protein. The homogenate was centrifuged as before and resuspended to a protein concentration of 333 µg/ml in binding buffer (Wash buffer + 0.1% BSA and 1.0µM final phosphoramidon) for an assay

volume of 50µg membrane protein/150ul binding buffer. Phosphoramidon was from SIGMA BIOCHEMICALS, St. Louis, MO (cat# R-7385).

Competition binding assays were performed at room temperature in Falcon 96 well round bottom polypropylene plates. Each assay well contained 150 µl of MCH receptor containing membranes prepared as described above, 50 µl ^{125}I-Tyr MCH, 50 µl binding buffer, and 2 µl test compound in DMSO. ^{125}I-Tyr MCH (specific activity = 2200 Ci/mMol) is purchased from NEN, Boston, MA (Cat # NEX 373) and was diluted in binding buffer to provide a final assay concentration of 30 pM.

Non-specific binding was defined as the binding measured in the presence of 1 µM unlabeled MCH. MCH is purchased from BACHEM U.S.A., King of Prussia, PA (cat # H-1482). Assay wells used to determine MCH binding contained 150 µl of MCH receptor containing membranes, 50 µl ^{125}I-Tyr MCH, 25 µl binding buffer, and 25 µl binding buffer.

Assay plates were incubated for 1 hour at room temperature. Membranes were harvested onto WALLAC™ glass fiber filters (PERKIN-ELMER, Gaithersburg, MD) which were pre-soaked with 1.0% PEI (polyethyleneimine) for 2 hours prior to use. Filters were allowed to dry overnight, and then counted in a WALLAC 1205 BETA PLATE counter after addition of WALLAC BETA SCINT™ scintillation fluid.

For saturation binding, the concentration of ^{125}I-Tyr MCH was varied from 7 to 1,000 pM. Typically, 11 concentration points were collected per saturation binding curve. Equilibrium binding parameters were determined by fitting the allosteric Hill equation to the measured values with the aid of the computer program FitP™ (BIOSOFT, Ferguson, MO). For the compounds described herein, K_i values were below 1 micromolar, preferably below 500 nanomolar, more preferably below 100 nanomolar.

Example 3

Calcium Mobilization Assay

This Example illustrates a representative functional assay for monitoring the response of cells expressing melanin concentrating hormone receptors to melanin concentrating hormone. This assay can also be used to determine if test compounds act as agonists or antagonists of melanin concentrating hormone receptors.

Chinese Hamster Ovary (CHO) cells (American Type Culture Collection; Manassas, VA) were stably transfected with the MCH expression vector described in Example 2 via calcium

phosphate precipitation, and were grown to a density of 15,000 cells/well in FALCON™ black-walled, clear-bottomed 96-well plates (#3904, BECTON-DICKINSON, Franklin Lakes, NJ) in Ham's F12 culture medium (MEDIATECH, Herndon, VA) supplemented with 10% fetal bovine serum, 25 mM HEPES and 500 µg/mL (active) G418. Prior to running the assay, the culture medium was emptied from the 96 well plates. Fluo-3 calcium sensitive dye (Molecular Probes, Eugene, OR) was added to each well (dye solution: 1 mg FLUO-3 AM, 440 µL DMSO and 440 µl 20% pluronic acid in DMSO, diluted 1:4, 50 µl diluted solution per well). Plates were covered with aluminum foil and incubated at 37°C for 1-2 hours. After the incubation, the dye was emptied from the plates, cells were washed once in 100 µl KRH buffer (0.05 mM KCl, 0.115 M NaCl, 9.6 mM NaH_2PO_4, 0.01 mM $MgSO_4$, 25 mM HEPES, pH 7.4) to remove excess dye; after washing, 80 µl KRH buffer was added to each well.

Fluorescence response was monitored upon the addition of either human MCH receptor or test compound by a FLIPR™ plate reader (Molecular Devices, Sunnyvale, CA) by excitation at 480 nM and emission at 530 nM.

In order to measure the ability of a test compound to antagonize the response of cells expressing MCH receptors to MCH, the EC_{50} of MCH was first determined. An additional 20 µl of KRH buffer and 1 µl DMSO was added to each well of cells, prepared as described above. 100 µl human MCH in KRH buffer was automatically transferred by the FLIPR instrument to each well. An 8-point concentration response curve, with final MCH concentrations of 1 nM to 3 µM, was used to determine MCH EC_{50}.

Test compounds were dissolved in DMSO, diluted in 20 µl KRH buffer, and added to cells prepared as described above. The 96 well plates containing prepared cells and test compounds were incubated in the dark, at room temperature for 0.5–6 hours. It is important that the incubation not continue beyond 6 hours. Just prior to determining the fluorescence response, 100 µl human MCH diluted in KRH buffer to 2 x EC_{50} was automatically added by the FLIPR instrument to each well of the 96 well plate for a final sample volume of 200 µl and a final MCH concentration of EC_{50}. The final concentration of test compounds in the assay wells was between 1 µM and 5 µM. Typically, cells exposed to one EC_{50} of MCH exhibit a fluorescence response of about 10,000 Relative Fluorescence Units. Antagonists of the MCH receptor exhibit a response that is significantly less than that of the control cells to the $p \leq 0.05$ level, as measured using a parametric test of statistical significance. Typically, antagonists of the MCH receptor decreased the fluorescence response by about 20%, preferably by about 50%, and most preferably by at least 80% as compared to matched controls.

The ability of a compound to act as an agonist of the MCH receptor was determined by measuring the fluorescence response of cells expressing MCH receptors, using the methods described above, in the absence of MCH. Compounds that cause cells to exhibit fluorescence above background are MCH receptor agonists. Compounds that induce no detectable increase in the basal activity of the MCH receptor have no detectable agonist activity and are preferred.

Example 4

MDCK Cytotoxicity Assay

This Example illustrates the evaluation of compound toxicity using a Madin Darby canine kidney (MDCK) cell cytoxicity assay.

1 μL of test compound is added to each well of a clear bottom 96-well plate (PACKARD, Meriden, CT) to give final concentration of compound in the assay of 10 micromolar, 100 micromolar or 200 micromolar. Solvent without test compound is added to control wells.

MDCK cells, ATCC no. CCL-34 (American Type Culture Collection, Manassas, VA), are maintained in sterile conditions following the instructions in the ATCC production information sheet. Confluent MDCK cells are trypsinized, harvested, and diluted to a concentration of 0.1 x 10^6 cells/ml with warm (37°C) medium (VITACELL Minimum Essential Medium Eagle, ATCC catalog # 30-2003). 100 μL of diluted cells is added to each well, except for five standard curve control wells that contain 100 μL of warm medium without cells. The plate is then incubated at 37°C under 95% O_2, 5% CO_2 for 2 hours with constant shaking. After incubation, 50 μL of mammalian cell lysis solution is added per well, the wells are covered with PACKARD TOPSEAL stickers, and plates are shaken at approximately 700 rpm on a suitable shaker for 2 minutes.

Compounds causing toxicity will decrease ATP production, relative to untreated cells. The PACKARD, (Meriden, CT) ATP-LITE-M Luminescent ATP detection kit, product no. 6016941, is generally used according to the manufacturer's instructions to measure ATP production in treated and untreated MDCK cells. PACKARD ATP LITE-M reagents are allowed to equilibrate to room temperature. Once equilibrated, the lyophilized substrate solution is reconstituted in 5.5 mL of substrate buffer solution (from kit). Lyophilized ATP standard solution is reconstituted in deionized water to give a 10 mM stock. For the five control wells, 10 μL of serially diluted PACKARD standard is added to each of the standard curve control wells to yield a final concentration in each subsequent well of 200 nM, 100 nM, 50 nM, 25 nM and 12.5

nM. PACKARD substrate solution (50 µL) is added to all wells, which are then covered, and the plates are shaken at approximately 700 rpm on a suitable shaker for 2 minutes. A white PACKARD sticker is attached to the bottom of each plate and samples are dark adapted by wrapping plates in foil and placing in the dark for 10 minutes. Luminescence is then measured at 22°C using a luminescence counter (*e.g.*, PACKARD TOPCOUNT Microplate Scintillation and Luminescence Counter or TECAN SPECTRAFLUOR PLUS), and ATP levels calculated from the standard curve. ATP levels in cells treated with test compound(s) are compared to the levels determined for untreated cells. Cells treated with 10 µM of a preferred test compound exhibit ATP levels that are at least 80%, preferably at least 90%, of the untreated cells. When a 100 µM concentration of the test compound is used, cells treated with preferred test compounds exhibit ATP levels that are at least 50%, preferably at least 80%, of the ATP levels detected in untreated cells.

From the foregoing it will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention.

Enumerated embodiments:

1. A method for treating obesity in a mammalian patient, comprising determining whether or not the patient carries a melanocortin 4 receptor (MC4R) mutation and, if the patient carries such a mutation, administering an amount of a non-toxic melanin concentrating hormone (MCH) receptor antagonist effective to reduce the body mass index of the patient upon repeated administration.

2. A method according to enumerated embodiment 1, wherein the MCH receptor antagonist has a molecular mass less than 700 a.m.u. and is nonpeptidic.

3. A method according to enumerated embodiment 1, wherein the MCH receptor antagonist has no detectable MCH receptor agonist activity.

4. A method according to enumerated embodiment 1, wherein the MCH receptor antagonist binds to an MCH receptor with a K_i that is less than 1 micromolar.

5. A method according to enumerated embodiment 1, wherein the MCH receptor antagonist binds to an MCH receptor with a K_i that is less than 100 nanomolar.

6. A method according to enumerated embodiment 1, wherein the MCH receptor antagonist is administered orally.

7. A method according to enumerated embodiment 1, wherein the MCH receptor antagonist is administered by injection.

8. A method according to enumerated embodiment 1, wherein the determination of whether or not the patient carries a MC4R mutation is performed via PCR using a sample of a tissue or body fluid obtained from the patient.

9. A method for treating obesity in a patient carrying a MC4R mutation, comprising administering an effective amount of a non-toxic MCH receptor antagonist to a patient previously determined to carry such a mutation.

10. A method according to enumerated embodiment 9, wherein the MCH receptor antagonist has a molecular mass less than 700 a.m.u. and is nonpeptidic.

11. A method according to enumerated embodiment 9, wherein the MCH receptor antagonist has no detectable MCH receptor agonist activity.

12. A method according to enumerated embodiment 9, wherein the MCH receptor antagonist binds to an MCH receptor with a K_i that is less than 1 micromolar.

13. A method according to enumerated embodiment 9, wherein the MCH receptor antagonist binds to an MCH receptor with a K_i that is less than 100 nanomolar.

14. A method according to enumerated embodiment 9, wherein the MCH receptor antagonist is administered orally.

15. A method according to enumerated embodiment 9, wherein the MCH receptor antagonist is administered by injection.

METHODS FOR TREATING OBESITY IN PATIENTS WITH MC4 RECEPTOR MUTATIONS

ABSTRACT

Methods are provided for treating health conditions associated with altered MC4 receptor activity with melanin concentrating hormone receptor antagonists. Such compounds may be used, for example, to treat obesity and overeating, and to reduce body mass index, in patients carrying MC4R mutations.

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